

Table 1. Demographic characteristics of the study population	
Age (years)	65.8 ± 1.2
Gender	
Male	50.0
Female	50.0
Education (years)	12.5 ± 1.0
Marital status	
Married	60.0
Single	40.0
Occupation	
Professional	30.0
Managerial	20.0
Technical	10.0
Service	20.0
Unemployed	20.0
Income (€ per month)	1,200 ± 200
Health status	
Good	70.0
Fair	20.0
Poor	10.0
Smoking status	
Smoker	30.0
Non-smoker	70.0
Alcohol consumption	
Regular	20.0
Occasional	30.0
Never	50.0
Comorbidities	
Hypertension	40.0
Diabetes	20.0
Heart disease	10.0
Stroke	10.0
Chronic kidney disease	5.0
Chronic liver disease	5.0
Chronic respiratory disease	10.0
Chronic pain	15.0
Depression	10.0
Anxiety	10.0
Other	10.0

In re Application of:

Divisional of

Group Art Unit: 1646

Examiner: Lazar Wesley, E.

PRELIMINARY AMENDMENT

Sir:

Prior to examining the above-identified application, please amend the application as follows:

Please amend the specification as follows:

Page 1, before line 3, insert

-- This is a Divisional of Application Serial No. 09/000,442, filed May 19, 1998 (now allowed), which is a 371 of PCT/AU96/00472, filed July 26, 1996. The disclosures of each of which are incorporated herein by reference. --

Page 10, lines 6-14, delete in their entirety, and insert therefor

-- **Figure 5A** provides a graph showing transient expression of PGKlacOcat in CHO/M(1)2lacIN + / - metal and + / - IPTG. CHO/M(1)2lacIN cells were transiently transfected with PGKlacOcat, the cells were incubated for 48 hours post transfection in medium + / - metal and + / - IPTG. Levels of chloramphenicol

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transferase (cat) protein were measured in cell extracts. Levels of cat were expressed relative to uninduced levels set at 100%. Repression of >95% of the PKLlacOcat gene were observed in the presence of metal, and IPTG led to a derepression indicating that the observed repression was specific to the lac repressor. --

Page 10, lines 16-25, delete in their entirety, and insert therefor

-- **Figure 5B** shows, diagrammatically, the incorporation of lacO sequences in CMVTf to generate CMVlacOTf. The 18 base ideal lacO sequence was used to replace 18 bases in the CMV promoter between the TATAA (SEQ ID NO:4) box and transcription start point (tsp), and a dimer of that sequence was inserted into the restriction site *Pme* I between the tsp and the ATG start codon. The replacement was achieved by incorporating the lacO sequence in an oligonucleotide then used as a primer in the Polymerase Chain Reaction (PCR). The insertion of the lacO dimer was done by ligation of a restriction enzyme fragment from the plasmid pOP (Hannan, G., et al 1994) into the *Pme* I site of PCMVTf. --

Page 10, lines 27-30, delete in their entirety, and insert therefor

-- **Figure 5C** provides a graph demonstrating the stable expression of CMVlacOTf in CHO/M(1)2lacIN. The plasmid pCMVlacOTf was transfected with CHO cells and stable transformants selected (labelled CHO/M(1)2lacIN cells). Cells from 2 clonal lines were grown for 12 hours in medium +/-metal and +/-IPTG, fresh medium added and the conditioned medium collected after another 24 hours. Tf levels were estimated (visually) on a Western blot and are expressed relative to levels in the absence of metal and IPTG

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which was set at 100%. Metal induced repression levels of >90% was observed in both clones and IPTG relieved most of that repression indicating its specificity. Levels of IPTG used (20mM) may have been sub-optimal thus explaining the incomplete derepression observed. --

Page 11, lines 1-7, delete in their entirety.

Page 15, lines 8-20, delete in their entirety, and insert therefor

**-- Example 1: The expression of transferrin or IGF-1 in CHO cells**

Work over the last few years has been conducted in order to gain an understanding of the growth requirements of CHO-K1 in serum free medium (Crowley, J., 1989, Gray, P.P. et al., 1990 and Bridges, M., PhD Thesis). Long term growth from liquid nitrogen to large scale may be obtained with insulin or insulin-like growth factor (IGF), transferrin and fibronectin or laminin as the only exogenous proteins. Selenium also needs to be added as a trace element. A defined serum-free (SF) medium was developed referred to as UNSWSF+ITS. Slight changes in growth characteristics of the CHO K1 cell line have occurred with different samples of CCL61 obtained over the years from American Type Culture Collection (ATCC). The current CHO K1 CCL61 stock obtained from ATCC in 1994 grows with a doubling time of around 17 hours in UNSWSF+ITS medium.

Page 15, lines 23-28, delete in their entirety, and insert therefor

-- The coding sequences for the IGF1 and transferring (Tf) genes, including the sequences for protein secretion, were isolated from a commercial human liver cDNA library (Clontech)

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using Polymerase Chain Reaction. Sequences 5' of the AUG start codon were modified to include an optimal translation initiation site (ACCATGA (SEQ ID NO:1) replacing AAGATGA (SEQ ID NO:2), Kozak, M., 1986). --

Page 18, lines 7-16, delete in their entirety, and insert therefor

-- Figure 4A shows that the presence of transferrin in defined medium, helped the long term growth and viability of CHO-K1 cells in culture. Figure 4B shows the final cell numbers and percentage viability of CHOSVLTf cells maintained from greater than 10 days in IS medium. It can be seen from Figure 4B that while the final cell densities for the three cell lines were similar, CHO-K1 growing on UNSWSF-ITS medium had a viability of 60%, similar to the CHOSVLTf cells expressing transferrin, while the viability of the CHO-K1 cells growing on the UNSWSF+IS medium was only 20%. This data shows the importance of having the CHO cells secreting transferrin, particularly for long term stable growth and viability. --

Page 19, lines 3-10, delete in their entirety and insert therefor

-- CHO cells were transfected using standard techniques with M(1)2lacIN and pSV2Neo. Cells were treated with 400  $\mu$ g/ml of G418 for 2 weeks and clonal cell lines resistant to G418 were selected. Clonal lines (designated CHO/R(5)4 and CHO/R(10)3) were obtained that produced low basal and high metal induced levels of repressor, as detected by Western blot. The repressor was shown to be biologically active by its ability to repress the

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lacO-containing plasmid PGKlacOcat (Hannan, G., et al., 1994) transiently introduced into the cells (Fig. 5A). --

Page 19, lines 13-20, delete in their entirety, and insert therefor

-- An ideal *lac* operator sequence (ATTGTGAGCGCTCACAAT (SEQ ID NO:3)) based on the bacterial operator and rules for acceptable sites of insertion within a given promoter have been described (Hu & Davidson, 1987). The bacterial *lac* repressor has a high association constant for the ideal *lac* operator sequence which is a rare sequence with only three copies found in various mammalian genomes (Simons et al., 1984), thus offering good specificity of regulation of the target gene and minimal effect on the host genes (Simons et al., 1984). --

Page 19, lines 21-28, delete in their entirety, and insert therefor

-- *Lac* operator sequences were inserted into the CMVTf gene to allow for repression by the *lac* repressor. One *lac* operator sequence (Fig. 5B) replaced promoter wild-type sequences between the TATAA (SEQ ID NO:4) box and the transcription start point and the other two were inserted between the transcription start point and the AUG start codon. Stable expression of this new construct (CMVlacOTf) in CHO cells already containing a stable inducible *lac* repressor gene (see example 3) was significantly shut down when repressor protein was present (i.e., the following metal induction) (Fig 5C). --

IN THE CLAIMS:

Please cancel Claims 1-6 and 17-21.

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Please amend the claims as follows:

Claim 7. (Amended) A method for the regulated growth of a mammalian host cell in a culture medium, comprising the step of:

culturing said mammalian host cell in said culture medium, wherein said host cell includes:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the host cell in said culture medium operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, operably linked to an inducible promoter sequence.

Claim 11. (Amended) A method according to Claim 10, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 14. (Amended) A method according to Claim 7, wherein the culture medium is protein/serum-free medium.

Claim 15. (Amended) A method according to Claim 7, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 16. (Amended) A method according to Claim 15, wherein the mammalian host cell is a CHO-K1 cell.

Claim 22. (Amended) A host cell including:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the

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host cell in a protein/serum-free culture medium operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, operably linked to an inducible promoter sequence.

Claim 26. (Amended) A host cell according to Claim 22, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 29. (Amended) A host cell according to Claim 22, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 30. (Amended) A host cell according to Claim 29, wherein the mammalian host cell is a CHO-K1 cell.

**REMARKS**

Claims 7-16 and 22-30 are directed to the invention which was non-elected in Parent Application Serial No. 09/000,442 (see the Office Action dated May 24, 2000 and Restriction Requirement dated June 26, 2000 in said Parent Application).

The specification has been amended to refer to Parent Application Serial No. 09/000,442, to correct typographical errors with respect to the figure designations and to insert sequence ID numbers therein.

Claims 6, 11, 14-16, 26 and 29-30 have been amended to correct their improper multiple dependency.

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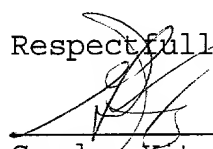
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The above amendments are consistent with the amendments made to Parent Application Serial No. 09/000,442.

Hence, the amendments to the specification and claims do not constitute new matter, and thus entry is respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

  
Gordon Kit

Registration No. 30,764

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Date: September 28, 2001



## A P P E N D I X

### Marked-Up Version of Changes

#### IN THE SPECIFICATION:

Page 1, before line 3, the following paragraph is being inserted

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"**Figure 5B** [5A] shows, diagrammatically, the incorporation of lacO sequences in CMVTf to generate CMVlacOTF. The 18 base ideal lacO sequence was used to replace 18 bases in the CMV promoter between the TATAA (SEQ ID NO:4) box and transcription start point (tsp), and a dimer of that sequence was inserted into the restriction site *Pme* I between the tsp and the ATG start codon. The replacement was achieved by incorporating the lacO sequence in an oligonucleotide then used as a primer in the Polymerase Chain

Reaction (PCR). The insertion of the lacO dimer was done by ligation of a restriction enzyme fragment from the plasmid pOP (Hannan, G., et al 1994) into the *Pme* I site of PCMVtF."

Page 10, lines 27-30, and Page 11, lines 1-7, are changed as follows:

"**Figure 5C [5B]** provides a graph demonstrating the stable expression of CMVlacOTf in CHO/M(1)2lacIN. The plasmid pCMVlacOTf was transfected with CHO cells and stable transformants selected (labelled CHO/M(1)2lacIN cells). Cells from 2 clonal lines were grown for 12 hours in medium +/-metal and +/-IPTG, fresh medium added and the conditioned medium collected after another 24 hours. Tf levels were estimated (visually) on a Western blot and are expressed relative to levels in the absence of metal and IPTG which was set at 100%. Metal induced repression levels of >90% was observed in both clones and IPTG relieved most of that repression indicating its specificity. Levels of IPTG used (20mM) may have been sub-optimal thus explaining the incomplete derepression observed."

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**"Example 1: The expression of transferrin or IGF-1 in CHO cells**

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CHO/R(10)3) were obtained that produced low basal and high metal induced levels of repressor, as detected by Western blot. The repressor was shown to be biologically active by its ability to repress the lacO-containing plasmid PGKlacOcat (Hannan, G., et al., 1994) transiently introduced into the cells [(Fig. 5)] (Fig. 5A)."

Page 19, lines 13-20, are changed as follows:

"An ideal lac operator sequence (ATTGTGAGCGCTCACAAT (SEQ ID NO:3)) based on the bacterial operator and rules for acceptable sites of insertion within a given promoter have been described (Hu & Davidson, 1987). The bacterial lac repressor has a high association constant for the ideal lac operator sequence which is a rare sequence with only three copies found in various mammalian genomes (Simons et al., 1984), thus offering good specificity of regulation of the target gene and minimal effect on the host genes (Simons et al., 1984)."

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**IN THE CLAIMS:**

Claims 1-6 and 17-21 are being cancelled.

The claims are amended as follows:

Claim 7. (Amended) A method for the regulated growth of a mammalian host cell in a culture medium, comprising the step of:

culturing said mammalian host cell in said culture medium, wherein said host cell includes:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the host cell in said culture medium [expressibly] operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, [expressibly] operably linked to an inducible promoter sequence.

Claim 11. (Amended) A method according to [any one of the preceding claims] Claim 10, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 14. (Amended) A method according to [any one of the preceding claims] Claim 7, wherein the culture medium is protein/serum-free medium.

Claim 15. (Amended) A method according to [any one of the preceding claims] Claim 7, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 16. (Amended) A method according to [any one of the preceding claims] Claim 15, wherein the mammalian host cell is a CHO-K1 cell.

Claim 22. (Amended) A host cell including:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the host cell in a protein/serum-free culture medium [expressibly] operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, [expressibly] operably linked to an inducible promoter sequence.

Claim 26. (Amended) A host cell according to [any one of claims 17 to 25] Claim 22, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 29. (Amended) A host cell according to [any one of claims 17 to 28] Claim 22, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 30. (Amended) A host cell according to [any one of claims 17 to 29] Claim 29, wherein the mammalian host cell is a CHO-K1 cell.